

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
25 May 2001 (25.05.2001)

PCT

(10) International Publication Number
WO 01/35913 A1

(51) International Patent Classification⁷: **A61K 7/06**

(21) International Application Number: **PCT/KR00/01281**

(22) International Filing Date:
9 November 2000 (09.11.2000)

(25) Filing Language: **Korean**

(26) Publication Language: **English**

(30) Priority Data:
1999/51646 19 November 1999 (19.11.1999) KR
2000/14837 23 March 2000 (23.03.2000) KR

(71) Applicant: **LG CHEMICAL CO., LTD.** [KR/KR]; 20,
Youido-dong, Youngdeungpo-ku, Seoul 150-010 (KR).

(72) Inventors: **KIM, Sang, Nyun**; 106 Dong 102 Ho, Sejong
Apartment, 462-5, Jeonmin-dong, Yuseong-ku, Daejeon
305-390 (KR). **AHN, Ho, Jeong**; 107 Dong 1106 Ho,
Sejong Apartment, 462-5, Jeonmin-dong, Yuseong-ku,
Daejeon 305-390 (KR). **KIM, Myung, Kee**; 101 Dong
1505 Ho, Hansol Apartment, Songkang-dong, Yuseong-ku,
Daejeon 305-503 (KR). **KIM, Jong, Il**; 105 Dong 501 Ho,
Narae Apartment, Jeonmin-dong, Yuseong-ku, Daejeon
305-390 (KR). **KIM, Jung, Hun**; 1 Dong 103 Ho, LG
Apartment, Doryong-dong, Yuseong-ku, Daejeon 305-340
(KR). **LEE, Chang, Woo**; 106 Dong 404 Ho, Sangloksoo
Apartment, Mannyon-dong, Seo-ku, Daejeon 302-150
(KR). **LEE, Min, Ho**; 1 Dong 105 Ho, LG Apartment, Do-
ryong-dong, Yuseong-ku, Daejeon 305-340 (KR). **KIM,**
Chang, Deok; 1 Dong 402 Ho, LG Apartment, 386-4,

Doryong-dong, Yuseong-ku, Daejeon 305-340 (KR). **CHO, Ho, Song**; 110 Dong 1405 Ho, Hanaro Apartment,
Walpyung-dong, Seo-ku, Daejeon 302-280 (KR). **KIM,**
Hyun, Sik; 8 Dong 503 Ho, LG Apartment, 386-4, Do-
ryong-dong, Yuseong-ku, Daejeon 305-340 (KR). **JUNG,**
Min, Hwan; 5 Dong 401 Ho, LG Apartment, 386-4, Do-
ryong-dong, Yuseong-ku, Daejeon 305-340 (KR). **KIM,**
Seung, Jin; 6/1, 270-27, Kaebong-dong, Kuro-ku, Seoul
152-093 (KR).

(74) Agent: **LEE, Byung, Hyun**; No. 906, Poong-Lim Bldg.,
823-1, Yeoksam-dong, Kangnam-ku, Seoul 135-784 (KR).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, KE, KG, KP, KZ, LC, LK, LR, LS, LT,
LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ,
PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT,
TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **USE OF A NONIMMUNOSUPPRESSIVE CYCLOSPORIN A DERIVATIVE FOR HAIR GROWTH**

(57) Abstract: The present invention relates to agents for treating alopecia and stimulating hair growth comprising an active ingredient of nonimmunosuppressive [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A having superior hair growth-promoting effect, wherein the hydroxyl group is added to the γ carbon position of No.4 N-methyl-L-leucine of cyclosporin A by the microorganism.

WO 01/35913 A1

USE OF A NONIMMUNOSUPPRESSIVE CYCLOSPORIN A DERIVATIVE
FOR HAIR GROWTH

Technical Field

5 The present invention relates to a hair growth promoter comprising a cyclosporin A derivative as an active ingredient which has much low degree of immunosuppression and maintains a good hair growth.

Background Art

10 Approximately 100,000 to 150,000 hairs exist in human body, each hair growing and falling out through different cycles of anagen, catagen, and telogen. These cycles are repeated through 3 to 6 years resulting in that average 50 to 100 hairs are normally fallen per day. Alopecia generally means that hair proportion of
15 anagen among these cycles is lessened and hairs of catagen or telogen are increased so that the number of fallen hairs is abnormally increased.

Opinions of poor blood circulation, excessive male sex hormone functioning, seborrhea, scalp function
20 deterioration by peroxides, bacteria, etc., hereditary factors, aging stresses, etc have been argued as reasons for the hair loss. However, explicit reasons for hair loss have not been identified up to now, recent trends are that population worrying about hair loss caused by
25 stress increase due to dietary habit change, social environment, etc. is being increased, its age is also being lowered, and female hair loss population is also being increased.

A preparation containing minoxidil which is most
30 widely used until now in the treatment or prevention of this alopecia is one of two hair revitalization ingredients which have received a permission of the U.S. Food and Drug Administration. Minoxidil has become a medication that is now more famous as a hair growth

promoter since trichogenous (i.e., promoting hair growth) phenomena occur due to side effects in the application although minoxidil was a high blood pressure treating agent that had been originally developed for the purpose of blood pressure drop. Although trichogenous mechanisms of minoxidil are not exactly discovered, blood flow increase through vasodilatation effects is thought to help supply nutrition to a hair root, thus promoting the hair growth.

This blood flow increase model is indirectly proved by the recent report that minoxidil increases the of VEGF (vascular endothelial growth factor), a growth factor related with vasodilatation, at dermal papilla which is a major cell forming a hair root (Br. J. of Dermatol., 1998; 138; 407 ~ 411). Furthermore, dermal papilla cell activation of a hair roots (Skin Pharmacol., 1996; 9; 3 ~ 8) and the research report showing that hair follicle growth is promoted in the hair follicle tissue culture (J. Invest. Dermatol., 1989; 92; 315 ~ 320), etc. besides vasodilatation effects in the hair growth-stimulating effect mechanism of minoxidil suggest that minoxidil acts as a direct growth factor in a hair root.

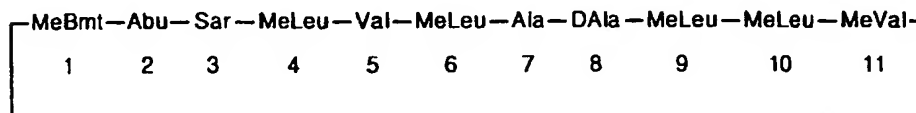
Additionally, the Merck Corporation's recently commercially available Propecia, principal ingredient being finasteride, inhibits the transformation of male sex hormone testosterone into dehydrotestosterone, a more potent male sex hormone.

Although the finasteride, which is now being commercially available after 1 mg tablet was received usage permission from Food and Drug Administration in December 1997, showed notable effects as a result of clinical tests partial side effects of male sex function suppression have also been reported (J. Am. Acad. Dermatol., 1998; 39; 578 ~ 589). However, searches and studies on superior hair growth promoter are actively

being pursued since the medication like minoxidil does not have excellent clinical effects either and due to concern over side effects.

It has been reported that cyclosporin is not only a representative immunosuppressant, but it also brings about various physiological effects such as nephrotoxicity, hepatotoxicity, high blood pressure, hair growth-stimulating effect, gingival over growth, and antimicrobial effects against viruses, fungi, and protozoa (Advances in Pharmacol., 1996; 35; 114 ~ 246 and Drug Safety, 1994; 10 310 ~ 317). A representative cyclosporin A is shown in the following Structural Formula 1 as a cyclic peptide with 11 amino acids comprising various N-methyl amino acids, and D-alanine at the No. 8 position.

[Structural Formula 1]



where MeBmt is N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine; Abu is L- α aminobutyric acid; Sar is Sarcosine; MeLeu is N-methyl-L-leucine; Val is L-valine; Ala is L-alanine; DAla is D-alanine; and MeVal is N-methyl-L-Valine.

Furthermore, the amino acid form of the above cyclosporin A is L-configuration, unless otherwise specified. Residue numbers of amino acids is assigned 1 for MeBmt and clock-wisely, 11 for the last MeVal (N-methyl-L-valine) as shown in Structural Formula 1. For nomenclature of cyclosporin A derivatives, only the substituted residue is expressed, for example, when the cyclosporin A derivative, in which N-methyl-L-leucine, at No. 4 is substituted by γ -hydroxy-N-methyl-L-leucine, it is expressed by [γ -hydroxy-N-methyl-L-leucine⁴]

cyclosporin A. Abbreviation of amino acid commonly used is also employed in specification, for example, MeLeu representing N-methyl-L-leucine, MeIle representing N-methyl-L-isoleucine, MeVal representing N-methyl-L-valine, MeAla representing N-methyl-L-alanine, MeNva representing N-methyl-L-norvaline, MePhe representing N-methyl-L-phenylalanine, Pip representing L-pipecolic acid, Leu representing L-leucine, Ile representing L-isoleucine, Sar representing sarcosine.

10 Possibilities for the development of cyclosporin as a new hair growth stimulator using excessive hair growth side effects have been reviewed in a variety of studies. Among them, animal hair growth-stimulating tests (Arch. Dermatol. Res., 1996; 288; 408 ~ 410),
15 human alopecia areata (J. Am. Acad. Dermatol., 1990; 22; 242 ~ 250), human male pattern alopecia (J. Am. Acad. Dermatol., 1990; 22; 251 ~ 253 and Skin Pharmacol., 1994; 7; 101 ~ 104), and protection from chemotherapy-induced alopecia (Clin. Lab. Invest., 1995; 190; 192 ~
20 196 and J. Pathol., 1997; 150; 1433 ~ 1441) have been reported and have shown about 100 times superior effects than minoxidil when compared to as the results of mouse backside test. Various patents have been applied as the results of efforts to utilize cyclosporin as a treatment
25 for male pattern alopecia based on these results.

For example, hair-growth promoters using these cyclosporin and derivatives in Japanese Laid-open Patent Publication Nos. Showa 60-243008, Showa 62-19512, and Showa 62-19513, cyclosporin derivatives with No. 8
30 position changed (European Laid-open Patent Publication No. 0414632B1), and isocyclosporin (World Laid-open Patent Publication No. 93/17039), etc., are provided and hair-growth promoters in which transdermal absorption of cyclosporin is superior are also provided in U.S. Patent
35 No. 5,807,820 and U.K. Patent No. 2,218,334A. However, there are many limits in a practical application due to

the severe side effects of immunosuppression although all cyclosporin groups used here have superior trichogenous effects for alopecia. Recently, an application for patent concerning the hair growth using nonimmunosuppressive cyclosporin derivatives in WO 051558A1 is made. However, it does not include the structure of the present [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A.

Disclosure of the Invention

10 In view of the forgoing problem in the prior art, an object of the present invention is to provide nonimmunosuppressive cyclosporin A derivatives of which the hair growth-stimulating effects are maintained while a degree of immunosuppression is lost, through various molecular changes of cyclosporin molecule, on the basis of the current discoveries that the hair growth-stimulating effects do not necessarily correlate with the immunosuppressive activity of cyclosporin molecules (Iwabuchi et al., J. Dermatol. Sci., 1995; 9; 64 - 69).

20 As an approach similar to this, studies on derivatives in which suppression of the human immunodeficiency virus (HIV) is maintained while a degree of immunosuppression is decreased are actively being pursued, particularly with derivatives in which the MeLeu group at the position 4 is replaced by a variety of N-methylated amino acid, for example γ -hydroxy-methylleucine, methylisoleucine, methylvaline, methylthreonine, methylalanine, which have been reported in patents (European Patent No. 484281 A2, U.S. Patent No. 5,767,069, U.S. Patent No. 5,981,479) and literature (J. Virol., 1995; 69: 2451-2461, J. Antibiotics, 1996; 49: 781-787) as new anti-HIV preparations.

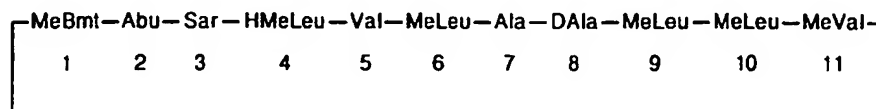
30 It is an object of the present invention to provide a new hair growth promoter of [γ -hydroxy-MeLeu⁴] cyclosporin A in which degree of immunosuppression is

35

lost while hair growth stimulating effects are uniquely maintained by evaluating trichogenous tests and degree of immunosuppression on various derivatives including those in which original amino acid of No. 4, N-methyl-L-leucine, is substituted with the similarly structured γ -hydroxy-N-methyl-L-leucine, methyl-isoleucine, methylvaline, leucine, isoleucine, methylalanine, methylphenylalanine, methylnorvaline, pipecolic acid, or sarcosine.

In order to accomplish the above objects, the present invention provides a hair growth promoter comprising an active ingredient of [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A represented as in the following Chemical Formula 1 in which hydroxyl group is added to γ carbon position of No. 4 N-methyl-L-leucine of cyclosporin A by the microbiological metabolic procedure.

[Chemical Formula 1]



where MeBmt is N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine; Abu is L- α aminobutyric acid; Sar is Sarcosine; HMeLeu is γ -hydroxy-N-methyl-L-leucine; Val is L-valine; MeLeu is N-methyl-L-leucine; Ala is L-alanine; DAla is D-alanine; and MeVal is N-methyl-L-Valine.

Furthermore, the present invention provides a hair growth promoter, wherein the composition is prepared in a form of liquid formulation, spray, gel, paste, emulsion, cream, conditioner, or shampoo.

Brief Description of the Drawings

The above objects, and other features and

advantages of the present invention will become more apparent after a reading of the following detailed description when taken in conjunction with the drawings, in which:

5 FIG. 1 is the high pressure liquid chromatography results for cyclosporin A derivative transformed by microorganisms and cyclosporin A that is not transformed by microorganisms;

10 FIG. 2 is the high pressure liquid chromatography results obtained by injecting again after purifying [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A;

 FIG. 3 is the high pressure liquid chromatography results obtained by simultaneously injecting cyclosporin A and [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A;

15 FIG. 4 is the Mass Spectroscopy results using an Electro-Spray Ionization method of cyclosporin A wherein [M(cyclosporin A) + H] peak is at m/z of 1202.8;

20 FIG. 5 is the LCQ Mass Spectrometer results using an Electro-Spray Ionization method of [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A wherein [M(cyclosporin derivative) + H] peak is at m/z of 1218.5 at which molecular weight of 16 is increased compared to cyclosporin;

25 FIG. 6 is the Collision Induced Dissociation test results of cyclosporin A;

 FIG. 7 is a table in which the Collision Induced Dissociation test results of cyclosporin A and [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A are compared by the fragment ion mass spectrum;

30 FIG. 8 is a ¹³C-Nuclear Magnetic Resonance spectrum of cyclosporin A;

 FIG. 9 is a ¹³C-Nuclear Magnetic Resonance spectrum of [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A;

35 FIG. 10 is the DEPT (distortionless enhancement by polarization transfer) test results on a new 69 ppm peak carbon of [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A

molecule wherein the new carbon peak with hydroxyl group attached is quaternary carbon;

FIG. 11 illustrates a ^{13}C -Nuclear Magnetic Resonance spectrum of cyclosporin A and [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A showing a peak removed by microorganisms is near 25 ppm;

FIG. 12 illustrates the DEPT (distortionless enhancement by polarization transfer) test results on the vicinity of the removed peak among cyclosporin A molecules, showing that the four peaks represent methine carbons in four N-methyl-L-leucines of cyclosporin A;

FIG. 13 illustrates the DEPT (distortionless enhancement by polarization transfer) test results of [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A molecules, thus representing, when compared to FIG. 12, that the peak of a methine carbon in N-methyl-L-leucine is removed;

FIG. 14 is a photograph evaluating hair growth effects of cyclosporin A and [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A using C57BL/6 mouse, particularly showing a control group;

FIG. 15 is a photograph evaluating hair growth effects of cyclosporin A and [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A using C57BL/6 mouse, particularly showing a group to which [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A is applied;

FIG. 16 is a photograph evaluating hair growth effects of cyclosporin A and [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A using C57BL/6 mouse, particularly showing a group to which cyclosporin A is applied;

FIG. 17 is a photograph evaluating hair growth effects of cyclosporin A, [methylisoleucine⁴] cyclosporin A, [methylvaline⁴] cyclosporin A, [leucine⁴] cyclosporin A, [isoleucine⁴] cyclosporin A and [methylalanine⁴] cyclosporin A using C57BL/6 mouse, particularly showing a control group;

FIG. 18 is a photograph evaluating hair growth effects of cyclosporin A, [methylisoleucine⁴] cyclosporin A, [methylvaline⁴] cyclosporin A, [leucine⁴] cyclosporin A, [isoleucine⁴] cyclosporin A and [methylalanine⁴] cyclosporin A using C57BL/6 mouse, particularly showing a group to which cyclosporin A is applied;

FIG. 19 is a photograph evaluating hair growth of cyclosporin A, [methylisoleucine⁴] cyclosporin A, [methylvaline⁴] cyclosporin A, [leucine⁴] cyclosporin A, [isoleucine⁴] cyclosporin A and [methylalanine⁴] cyclosporin A using C57BL/6 mouse, particularly showing a group to which [methylisoleucine⁴] cyclosporin A is applied;

FIG. 20 is a photograph evaluating hair growth effects of cyclosporin A, [methylisoleucine⁴] cyclosporin A, [methylvaline⁴] cyclosporin A, [leucine⁴] cyclosporin A, [isoleucine⁴] cyclosporin A and [methylalanine⁴] cyclosporin A using C57BL/6 mouse, particularly showing a group to which [methylvaline⁴] cyclosporin A is applied;

FIG. 21 is a photograph evaluating hair growth effects of cyclosporin A, [methylisoleucine⁴] cyclosporin A, [methylvaline⁴] cyclosporin A, [leucine⁴] cyclosporin A, [isoleucine⁴] cyclosporin A and [methylalanine⁴] cyclosporin A using C57BL/6 mouse, particularly showing a group to which [leucine⁴] cyclosporin A is applied;

FIG. 22 is a photograph evaluating hair growth effects of cyclosporin A, [methylisoleucine⁴] cyclosporin A, [methylvaline⁴] cyclosporin A, [leucine⁴] cyclosporin A, [isoleucine⁴] cyclosporin A and [methylalanine⁴] cyclosporin A using C57BL/6 mouse, particularly showing a group to which [isoleucine⁴] cyclosporin A is applied;

FIG. 23 is a photograph evaluating hair growth effects of cyclosporin A, [methylisoleucine⁴] cyclosporin A, [methylvaline⁴] cyclosporin A, [leucine⁴] cyclosporin A, [isoleucine⁴] cyclosporin A and [methylalanine⁴] cyclosporin A using C57BL/6 mouse, particularly showing a group to which [isoleucine⁴] cyclosporin A is applied;

cyclosporin A using C57BL/6 mouse, particularly showing a group to which [methylalanine⁴] cyclosporin A is applied;

5 FIG. 24 is a photograph evaluating hair growth effects of cyclosporin A, [methylphenylalanine⁴] cyclosporin A, [pipecolic acid⁴] cyclosporin A, [sarcosine⁴] cyclosporin A, and [methylnorvaline⁴] cyclosporin A using C57BL/6 mouse, particularly showing a control group;

10 FIG. 25 is a photograph evaluating hair growth effects of cyclosporin A, [methylphenylalanine⁴] cyclosporin A, [pipecolic acid⁴] cyclosporin A, [sarcosine⁴] cyclosporin A, and [methylnorvaline⁴] cyclosporin A using C57BL/6 mouse, particularly showing a group to which cyclosporin A is applied;

15 FIG. 26 is a photograph evaluating hair growth effects of cyclosporin A, [methylphenylalanine⁴] cyclosporin A, [pipecolic acid⁴] cyclosporin A, [sarcosine⁴] cyclosporin A, and [methylnorvaline⁴] cyclosporin A using C57BL/6 mouse, particularly showing a group to which [methylphenylalanine⁴] cyclosporin A is applied;

20 FIG. 27 is a photograph evaluating hair growth effects of cyclosporin A, [methylphenylalanine⁴] cyclosporin A, [pipecolic acid⁴] cyclosporin A, [sarcosine⁴] cyclosporin A, and [methylnorvaline⁴] cyclosporin A using C57BL/6 mouse, particularly showing a group to which [pipecolic acid⁴] cyclosporin A is applied;

25 FIG. 28 is a photograph evaluating hair growth effects of cyclosporin A, [methylphenylalanine⁴] cyclosporin A, [pipecolic acid⁴] cyclosporin A, [sarcosine⁴] cyclosporin A, and [methylnorvaline⁴] cyclosporin A using C57BL/6 mouse, particularly showing a group to which [sarcosine⁴] cyclosporin A is applied; and

35

FIG. 29 is a photograph evaluating hair growth effects of cyclosporin A, [methylphenylalanine⁴] cyclosporin A, [pipecolic acid⁴] cyclosporin A, [sarcosine⁴] cyclosporin A, and [methylnorvaline⁴] cyclosporin A using C57BL/6 mouse, particularly showing a group to which [methylnorvaline⁴] cyclosporin A is applied.

Best Mode for Carrying Out the Invention

In the following detailed description, only the preferred embodiments of the invention have been shown and described, simply by way of illustration of the best mode contemplated by the inventor(s) of carrying out the invention. As will be realized, the invention is capable of modification in various obvious respects, all without departing from the invention. Accordingly, the description is to be regarded as illustrative in nature, and not restrictive.

The present invention is described in detail as following:

The inventors have studied to discover a cyclosporin A derivative maintaining hair growth effects without immunosuppression in order to develop a new trichogenous ingredient. We carried out the hair growth evaluation tests by synthesizing and transforming cyclosporin A derivatives of the following REFERENCE EXAMPLES, as the result it was observed that most of effects of the derivatives were remarkably decreased compared to cyclosporin A before the transformation. However, it was observed that the hair growth effects of [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A transformed by microorganisms of the present invention were maintained as comparing with cyclosporin A.

REFERENCE EXAMPLES

<u>REFERENCE</u>	<u>EXAMPLE</u>	<u>1:</u>	<u>preparation</u>	<u>of</u>
------------------	----------------	-----------	--------------------	-----------

[methyloisoleucine⁴] cyclosporin A

After condensing decapeptide (H-Val-MeLeu-Ala-(D)Ala-MeLeu-MeLeu-MeVal-MeBmt(OAc)-Abu-Sar-OMe) with Boc-MeIle-OH using condensing reagents of benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium
5 hexafluorophosphate and dimethylaminopyridine, undeca-peptides were obtained. Its protecting groups were then removed by sodium hydroxide (NaOH) and trifluoroacetic acid (TFA). The resulting material is
10 subjected to the cyclization using benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate and dimethylaminopyridine to obtain a substituted cyclosporin A-acetate. The acetyl group is then removed by using sodium methoxide (NaOMe) to give
15 [methyloisoleucine⁴] cyclosporin A.

REFERENCE EXAMPLE 2: preparation of [MeVal⁴] cyclosporin A

[MeVal⁴] cyclosporin A was synthesized by the
20 preparation method of REFERENCE EXAMPLE 1 except that Boc-MeVal-OH was used instead of Boc-MeIle-OH.

REFERENCE EXAMPLE 3: preparation of [Leu⁴] cyclosporin A

[Leu⁴] cyclosporin A was synthesized by the
25 preparation method of REFERENCE EXAMPLE 1 except that Boc-Leu-OH was used instead of Boc-MeIle-OH.

REFERENCE EXAMPLE 4: preparation of [Ile⁴] cyclosporin A

[Ile⁴] cyclosporin A was synthesized by the
30 preparation method of REFERENCE EXAMPLE 1 except that Boc-Ile-OH was used instead of Boc-MeIle-OH.

REFERENCE EXAMPLE 5: preparation of [MeAla⁴]

cyclosporin A

[MeAla⁴] cyclosporin A was synthesized by the preparation method of REFERENCE EXAMPLE 1 except that Boc-MeAla-OH was used instead of Boc-MeIle-OH.

5 REFERENCE EXAMPLE 6: preparation of [MePhe⁴]
cyclosporin A

[MePhe⁴] cyclosporin A was synthesized by the preparation method of REFERENCE EXAMPLE 1 except that Boc-MePhe-OH was used instead of Boc-MeIle-OH.

10 REFERENCE EXAMPLE 7: preparation of [MeNva⁴]
cyclosporin A

[MeNva⁴] cyclosporin A was synthesized by the preparation method of REFERENCE EXAMPLE 1 except that Boc-MeNva-OH was used instead of Boc-MeIle-OH.

15 REFERENCE EXAMPLE 8: preparation of [Pip⁴]
cyclosporin A

[Pip⁴] cyclosporin A was synthesized by the preparation method of REFERENCE EXAMPLE 1 except that Boc-Pip-OH was used instead of Boc-MeIle-OH.

20 REFERENCE EXAMPLE 9: preparation of [Sar⁴]
cyclosporin A

[Sar⁴] cyclosporin A was synthesized by the preparation method of REFERENCE EXAMPLE 1 except that Boc-Sar-OH was used instead of Boc-MeIle-OH.

25 REFERENCE EXAMPLE 10: preparation of cyclosporin
A-acetate

After dissolving 3.6 g (30 mmol) of cyclosporin A into 100 ml of tetrahydrofuran, 1.5 equivalents of acetic anhydride, 1.5 equivalents of triethylamine, and 0.3 g
30 of dimethylaminopyridine were put into it. The mixture was refluxed for 18 hours. The solvent was distilled

under reduced pressure. The residue was dissolved into ethyl acetate and washed with water. The organic layer was removed and refined with chromatography to obtain 3.2 g of cyclosporin A-acetate.

5 REFERENCE EXAMPLE 11: preparation of seco-
cyclosporin undecapeptide (H-MeLeu-Val-MeLeu-Ala-D-Ala-
MeLeu-MeLeu-MeVal-MeBmt(OAc)-Abu-Sar-OMe)

 After dissolving 3.2 g of cyclosporin A-acetate
into 30 ml of dichloromethane, 2.5 equivalents of
10 trimethyloxonium tetrafluoroborate ($(\text{CH}_3)_3\text{O}^+\text{BF}_4^-$) were
put into it and agitated at the room temperature for 20
hours. Sodium methoxide dissolved in 1.2 equivalents of
methanol was added to the mixture and was agitated for
30 minutes. Then, by adding 10 ml of 1 mol sulfuric acid
15 aqueous solution and 10 ml of methanol, the mixture was
acid hydrolyzed for 15 minutes. After distilling the
solvent under reduced pressure and refining with
chromatography, 2.0 g of seco-cyclosporin undecapeptide
was obtained (Wenger, The European Peptide Society,
20 1998; 173 ~ 177).

REFERENCE EXAMPLE 12: preparation of [Des-MeLeu⁴]-
cyclosporin A-acetate

 After removing N-methyl-L-leucine from the above
seco-cyclosporin undecapeptide by the Edman method (Eur.
25 J. Biochem., 1967; 1; 80), [Des-MeLeu⁴] cyclosporin A-
acetate was obtained by the cyclization.

REFERENCE EXAMPLE 13: preparation of [Des-MeLeu⁴]-
cyclosporin A

 Sodium methoxide (NaOCH_3) dissolved in methanol was
30 added to [Des-MeLeu⁴]-cyclosporin A-acetate. The mixture
was agitated for 3 hours and acidified by acetic acid.
After distilling the solvent under reduced pressure
and refining, [Des-MeLeu⁴]-cyclosporin A was obtained.

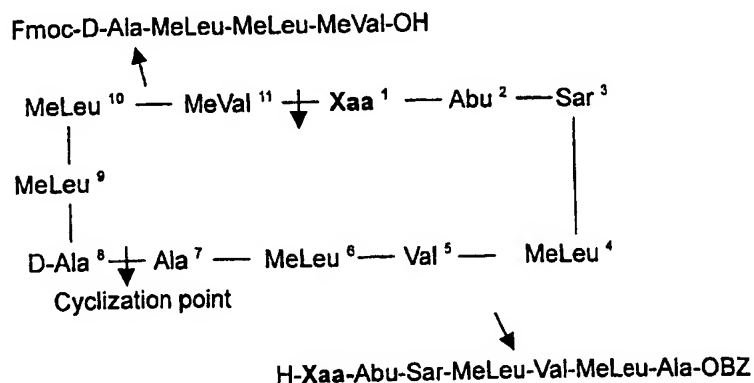
REFERENCE EXAMPLE 14: preparation of [Des-MeLeu⁴, Des-Val⁵]-cyclosporin A-acetate

[Des-MeLeu⁴, Des-Val⁵] cyclosporin A-acetate was obtained by the same synthesizing method of [Des-MeLeu⁴] cyclosporin A.

REFERENCE EXAMPLE 15: preparation of [Xaal]-cyclosporin A

Tetrapeptide (Fmoc-D-Ala-MeLeu-MeLeu-MeVal-OH) and 6 types of heptapeptide (H-Xaa-Abu-Sar-MeLeu-Val-MeLeu-Ala-Obzl) substituted with other amino acids instead of MeBmt were 4 + 7 fragment condensed using a condensing reagent of BOP reagent. The obtained undecapeptide was hydrolyzed to remove C-end benzyl group and N-end Fmoc-group. Cyclization was carried out using propylphosphonic anhydride and 4-(dimethylamino)pyridine. The final products were obtained by removing side chain protecting groups of Xaal if necessary. This reaction was represented in the following Reaction Formula 1.

[Reaction Formula 1]



where Xaal, a substituted amino acid, is Leu, Phe, MeLeu, Gly, Ala, or MeVal.

The present invention is described further in detail through the following EXAMPLES and TEST EXAMPLES. However, EXAMPLES are only for exemplifying the present invention, and the present invention is not limited to the EXAMPLES.

EXAMPLES

EXAMPLE 1: preparation of [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A

The preparation of [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A in which the hair growth effects are maintained after the transformation by microorganisms is described here.

Sebekia benihana KCTC 9173 was used for the production of the cyclosporin derivative. The culture medium consisted of 0.7% of glucose, 0.45% of yeast extract, 0.5% of malt extract, 1.0% of soluble starch, and 0.005% of calcium carbonate (CaCO₃), and the culture temperature of 27 °C was used (J. Antibiotics, 1996; 49; 781 ~ 787).

When using a fermentor, 4 l fermentor with the medium was used, in which 4 day old preculture in an Erlenmeyer flask was used as an inoculum.

After 24 hours of the main culture using the fermentor, cyclosporin dissolved in methanol was added to a medium in a concentration of 100 mg/l and further cultured for 72 hours.

In order to recover the samples after the culturing, total culture was extracted with ethylacetate of the same amount as the medium. The organic solvent layer was concentrated, and the concentrated samples were separated and collected by using a high pressure liquid chromatography. The liquid chromatography results showing cyclosporin A and [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A are represented in FIG.1 and the purified [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A

is represented in FIG. 2. The liquid chromatography results when simultaneously injecting cyclosporin A and the separated [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A are represented in FIG. 3.

5 Here, C-18 column was used for a separation purpose. The solvent system was that 100% solvent A were flown for 2 minutes and decreased to 60% in 4 minutes, and then to 39% in 60 minute to elute the samples with concomitant increase of solvent B. Then, the condition
10 returned to the original condition of 100% solvent A by 65 minutes. At this time, solvent A was 25% methanol aqueous solution and solvent B was 100% acetonitrile.

EXAMPLE 2: confirmation of structure of [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A

15 A LCQ mass spectrometer (Finnigan, CA) using the ESI (electro-spray ionization) method was used in order to analyze the structure of collected cyclosporin A derivatives. The tests were taken in a way of reciprocally comparing cyclosporin A and cyclosporin
20 derivatives.

In the Electro-Spray Ionization - Mass Spectrometer tests for confirming each molecular weights the cyclosporin A showed [M(cyclosporin) + H] peak at m/z 1202.8 (FIG. 4), and cyclosporin derivatives showed
25 [M(cyclosporin derivatives) + H] peak at m/z 1218.5 (Fig. 5), which indicates that the molecular weight of the derivative was increased by 16 compared to cyclosporin. Furthermore, the same tests were repeated with addition of sodium. As the result, [M(cyclosporin)
30 + Na] peak was detected at m/z 1224.7 in cyclosporin, and [M(cyclosporin derivatives) + Na] peak was observed at m/z 1240.7 in cyclosporin derivatives. From the above results, it could be presumed that hydroxyl group was added to cyclosporin molecules into cyclosporin A
35 derivatives (hydroxylation).

The CID (Collision Induced Dissociation) method was used in order to confirm the position of the amino acid where the hydroxylation was occurred among 11 amino acids of cyclosporin. After forming fragment ions by the collision induced dissociation method, the fragment ion pattern (FIG. 6) formed from cyclosporin A and fragment ion pattern formed from cyclosporin A derivatives were comparatively analyzed. When referring to the fragment ion patterns of FIG. 7, it was found that there was not any mass value changes in fragment ions of other amino acids, but the mass values of fragment ion peaks comprising No. 4 position amino acid (leucine) were increased by 16. Therefore, it could be known that the transformation was occurred at No. 4 position amino acid.

The nuclear magnetic resonance (ARX 300 MHz, Bruker, Germany) spectroscopy was additionally conducted in order to confirm that the added hydroxyl group was positioned at No. 4 amino acid, as was disclosed in the above test.

First, as comparing ^{13}C -Nuclear Magnetic Resonance spectrums of cyclosporin A (FIG. 8) and cyclosporin A derivatives (FIG. 9) a new peak (δ 69.00 ppm) representing chemical shift of carbon comprising the added hydroxyl groups was observed.

In order to locate the carbon of this peak DEPT (distortionless enhancement by polarization transfer) tests were carried out (FIG. 10). As the result, it was known that hydroxyl groups were attached to quaternary carbon, and this quaternization was made by adding hydroxyl group to γ -carbon position of No. 4 amino acids (hydroxylation). If quaternization had been occurred to α -carbon of No. 4 amino acid, the peak would have been shifted to down field near 90 ppm.

Referring to FIG. 11 to FIG. 13 wherein the DEPT test results are shown, it can be known that a peak

removed by microorganisms is in the vicinity of 25 ppm, and that one of 4 methine carbons of 4 N-methyl-L-leucine γ carbons in cyclosporin A molecules was removed.

Summing up, it could be known that hydroxyl group was added to No. 4 amino acid (N-methyl-L-leucine) from the result of mass spectrum method using electro-spray ionization and collision induced dissociation, and hydroxyl group was added to γ position carbon from the result of the nuclear magnetic resonance spectroscopy.

10 FORMULATIONS

FORMULATION 1: preparation of a hair revitalizing tonic containing cyclosporin A derivative

A hair-revitalizing tonic was prepared in 3 types of preparation forms represented in the following Table 1 by mixing, agitating, and completely dissolving each raw material.

[Table 1]

Ingredient (wt%)	Preparation Form		
	Form 1	Form 2	Form 3
Ethanol	40.0	40.0	40.0
[γ -hydroxy-N-methyl-L-leucine ⁴] cyclosporin A	0.1	1.0	8.0
Tocopherol acetic acid	0.1	0.1	0.1
Salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
Tween 20	0.5	0.5	0.5
Perfume	Approp. amount	Approp. amount	Approp. amount
Colorant	Approp. amount	Approp. amount	Approp. amount
Water	Balance	Balance	Balance

Formulation 2: preparation of a hair cream

containing cyclosporin A derivative

Oil soluble ingredients and water soluble ingredients were completely dissolved in each phase by separately mixing and heating to 80 °C in 3 types of preparation forms represented in the following Table 2. The two prepared phases at 80 °C were mixed and emulsified. After completing the emulsification and cooling to room temperature, a hair cream was prepared by adding and mixing perfume and colorant. An amount of water was added so that the total amount of two phases could be adjusted to 100 wt%.

[Table 2]

Ingredient (wt%)		Preparation Form		
		Form 1	Form 2	Form 3
Oil soluble raw materials	Paraffin	5.0	5.0	5.0
	Setostearylalcohol	5.5	5.5	5.5
	Petrolatum	5.5	5.5	5.5
	Glycerine -monostearate	3.0	3.0	3.0
	Polyoxyethylene octyldodecylether	3.0	3.0	3.0
	Propylparaben	0.3	0.3	0.3
	[γ -hydroxy-N-methyl-L-leucine ⁴] cyclosporin A	0.1	1.0	8.0
Water soluble raw materials	Glycerin	7.0	7.0	7.0
	Dipropyl glycol	20.0	20.0	20.0
	Polyethylene glycol	5.0	5.0	5.0
	Water	45.6	44.7	37.7
Perfume		Approp. amount	Approp. amount	Approp. amount
Colorant		Approp. amount	Approp. amount	Approp. amount

FORMULATION 3: preparation of a shampoo containing cyclosporin A derivative

Raw materials except perfume, colorant, and water in 3 types of preparation forms represented in the following Table 3 were mixed, until they were completely dissolved by heating while agitating. After cooling the mixture to the room temperature and adding perfume and colorant to it, finally water was added so that total composition content could be adjusted to 100 wt% to obtain a shampoo.

[Table 3]

Ingredient (wt%)	Preparation Form		
	Form 1	Form 2	Form 3
Sodium POE laurylsulfuric acid(30 wt% aqueous solution)	40.0	40.0	40.0
Coconut oil fatty acid Diethanolamide	3.0	3.0	3.0
1,2-propylene glycol	2.0	2.0	2.0
Methyl paraoxybenzoic acid	0.2	0.2	0.2
Ethanol	2.0	2.0	2.0
[γ -hydroxy-N-methyl-L-leucine ⁴] cyclosporin A	1.0	3.0	10.0
Salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
Perfume	Approp. amount	Approp. amount	Approp. amount
Colorant	Approp. amount	Approp. amount	Approp. amount
Water	Balance	Balance	Balance

FORMULATION 4: preparation of hair conditioner containing cyclosporin A derivative

Oil soluble materials and water soluble materials

among raw materials were separately mixed in 3 types of preparation forms represented in the following Table 4 and completely dissolved by heating up to 80 °C. The prepared mixtures of oil soluble raw materials and water soluble raw materials of 80 °C were mixed together and emulsified. After the emulsification and cooling to the room temperature, a hair conditioner was prepared by adding and mixing perfume and colorant.

The amount of water was added so that total composition content preparation could be adjusted to 100 wt%.

[Table 4]

Ingredient (wt%)		Preparation Form		
		Form 1	Form 2	Form 3
Oil soluble raw materials	Cetanol	3.0	3.0	3.0
	Self-emulsion type Glycerol-monostearate	2.0	2.0	2.0
	Squalene	10.0	10.0	10.0
	[γ -hydroxy-N-methyl-L-leucine ⁴] cyclosporin A	1.0	5.0	10.0
Water soluble raw materials	Propylene glycol	2.0	2.0	2.0
	Stearyldimethyl Benzylammonium chloride (25 wt% aqueous solution)	8.0	8.0	8.0
	Methyl paraoxybenzoic acid	0.2	0.2	0.2
	Salicylic acid	0.3	0.3	0.3
	L-menthol	0.3	0.3	0.3
	Water	73.2	69.2	64.2

Perfume	Approp. amount	Approp. amount	Approp. amount
Colorant	Approp. amount	Approp. amount	Approp. amount

TEST EXAMPLESTEST EXAMPLE 1: test of hair growth promoting effects of cyclosporin A derivatives

5 C57BL/6 mice (42 ~ 49 day old female) were used in the test of hair growth promoting effects.

First of all, several mice, after removing the back side hair using an electric shaver and weighing, were divided uniformly according to their weights. After one day of adaptation period, cyclosporin derivative
10 collected from HPLC of the above EXAMPLE 1 was applied on the area with hair removed in the amount of 100 μ l (0.1% w/v) once a day per each individual for 30 days. The degree of the hair growth were observed by naked eyes and photographed.

15 As seen in FIG. 14 to FIG 16, the remarkable hair growth promoting effects were shown when cyclosporin A (FIG. 16) and its derivative of [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A (FIG. 15) of EXAMPLE 1 were applied, compared to the control group (FIG. 14) on
20 which only vehicle were applied. Although the difference before and after the transformation was very much inappreciable, effects of the remaining derivatives (those mentioned in REFERENCE EXAMPLES 10 to 15) were as little as the control group having no effects.

25 In FIG. 17 to FIG. 23, the comparison tests of the other derivatives (REFERENCE EXAMPLES 1 to 5) similar to [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A of which the trichogenous effect was proved and cyclosporin A are shown. From the figures, it can be known that the
30 trichogenous effects of [methylisoleucine⁴] cyclosporin A (FIG. 19), [methylvaline⁴] cyclosporin A (FIG. 20),

[leucine⁴] cyclosporin A (FIG. 21), [isoleucine⁴] cyclosporin A (FIG. 22) and [methylalanine⁴] cyclosporin A (FIG. 23) are much poorer than cyclosporin A (FIG. 18). In the similarly designed experiment, [methylphenylalanine⁴] cyclosporin A (FIG. 26), [pipecolic acid⁴] cyclosporin A (FIG. 27), [sarcosine⁴] cyclosporin A (FIG. 28), and [methylnorvaline⁴] cyclosporin A (FIG. 29) are much poorer than cyclosporin A (FIG. 25). That is, it was noted that only [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A maintained the effects from test of the trichogenous evaluation of various types of cyclosporin A derivatives.

Upon observing the back conditions of mice during 30 days of test procedure, the appreciable skin irritations were not found from the control group and all treated groups.

TEST EXAMPLE 2: Immunosuppression tests of cyclosporin A derivatives

Using the MLR method (Mixed Allogenic Mixed Lymphocyte Reaction method) by mixing spleen cells of two different species of mouse (J. Antibiotics, 1994; 47; 208 ~ 215), the immunosuppression comparison test was carried out.

After mixing the equivalent numbers of BALB/c mouse spleen cells as reacting cells and mitomycin treated C57BL/6 mouse spleen cells as stimulating cells, the mixture was treated with cyclosporin A and [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A. And then, it was cultured in RPMI medium containing mercaptoethanol and 10% fetal bovine serum for 4 days. ³H-thymidine was added to the solution and cultured for further 4 hours. After culturing, IC₅₀ (μ g/ml) of each materials was calculated by liquid scintillation counteracting the amount of thymidine influxed into cells.

As the results of that, IC₅₀ (μ g/ml) of cyclosporin A

showed 0.034, 0.05, and 0.031 while $IC_{50}(\mu g/ml)$ of [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A showed 5.3, 6.8, and 5.3, indicating over 100 times decrease of immunosuppression. This was the similar level to the literature (J. Antibiotics, 1996, 49, 781 ~ 787, and J. Virol., 1995; 69; 2451 ~ 2461).

That is, it was found that [γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A in which hydroxyl group is added to the γ -carbon position of No. 4 N-methyl-L-leucine in cyclosporin A by the microorganism metabolism procedure not only had much lower degree of immunosuppression but also maintained superior hair growth effects compared to non-transformed cyclosporin A.

Trichogenous tonic, hair cream, hair conditioner, and hair shampoo which are commercially much in use, were prepared according to the present invention although the various preparation forms such as liquid formulation, spray, gel, paste, emulsion, cream, conditioner, shampoo, etc. are possibly be made by applying the result of the present invention. From the animal evaluation test of the TEST EXAMPLE 1 it was confirmed that treating group of the present invention had superior trichogenous effects than control group.

Industrial Applicability

A hair growth promoter comprising an active ingredient of cyclosporin A derivatives of the present invention has a much lower degree of immunosuppression compared to cyclosporin A before the transformation while maintaining the excellent hair growth effects leading the superior trichogenous effects.

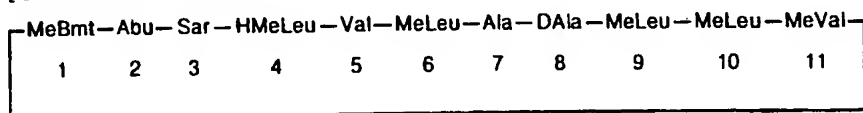
Although the preferred embodiments of the invention have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the

invention as disclosed in the accompanying claims.

WHAT IS CLAIMED IS:

1. A hair growth promoter comprising an active ingredient of γ -hydroxy-N-methyl-L-leucine⁴] cyclosporin A represented in the following Chemical Formula 1 having non-immunosuppressive activity and superior hair growth stimulating effects:

[Chemical Formula 1]



- where MeBmt is N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine; Abu is L- α aminobutyric acid; Sar is Sarcosine; HMeLeu is γ -hydroxy-N-methyl-L-leucine; Val is L-valine; MeLeu is N-methyl-L-leucine; Ala is L-alanine; DAla is D-alanine; and MeVal is N-methyl-L-Valine.

2. The hair growth promoter in accordance with claim 1, which is prepared in one or more forms selected from the group consisting of liquid formulation, spray, gel, paste, emulsion, cream, conditioner, and shampoo.

1/17

FIGURE

FIG. 1

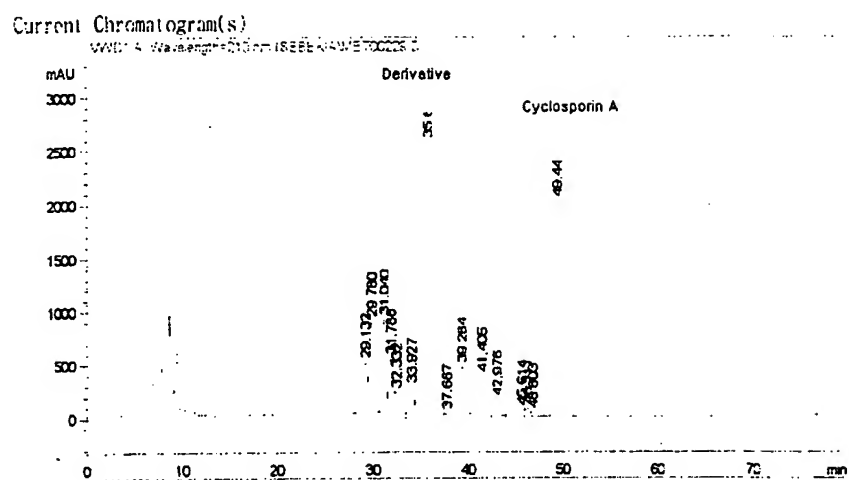
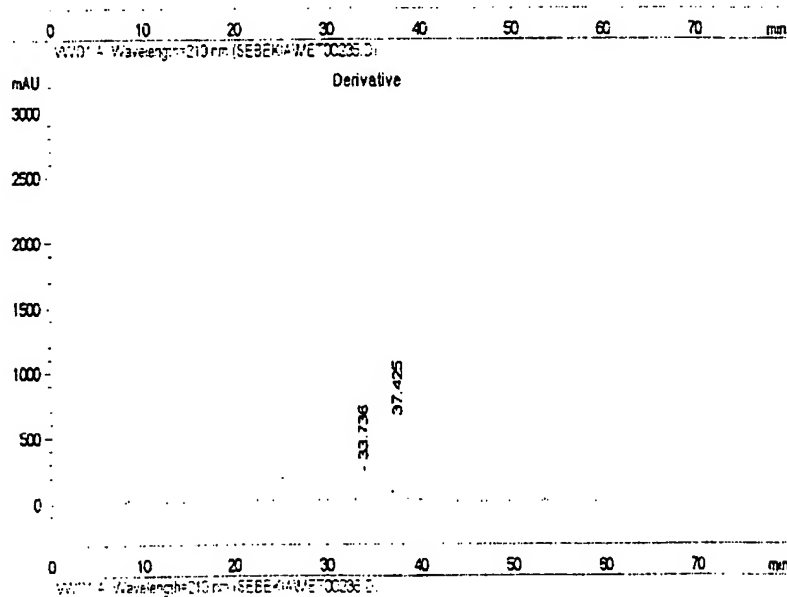


FIG. 2



BEST AVAILABLE COPY

2/17

FIG. 3

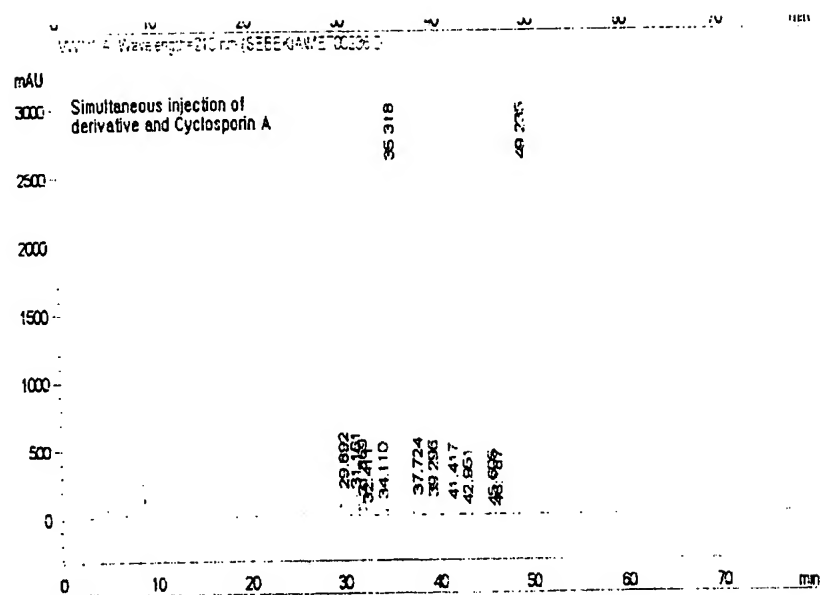
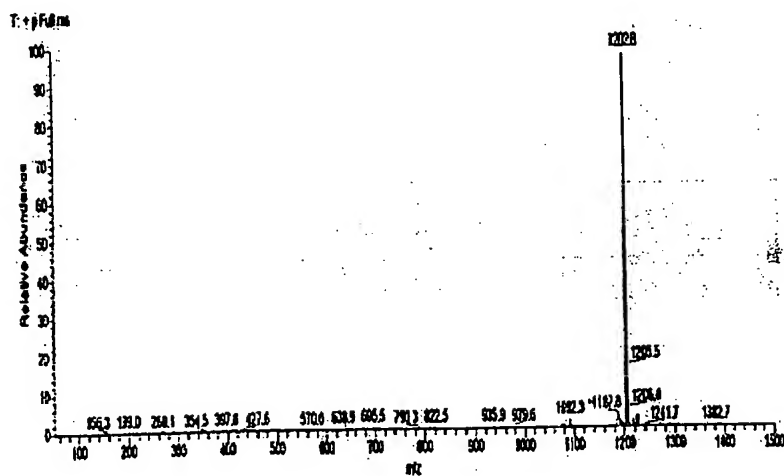


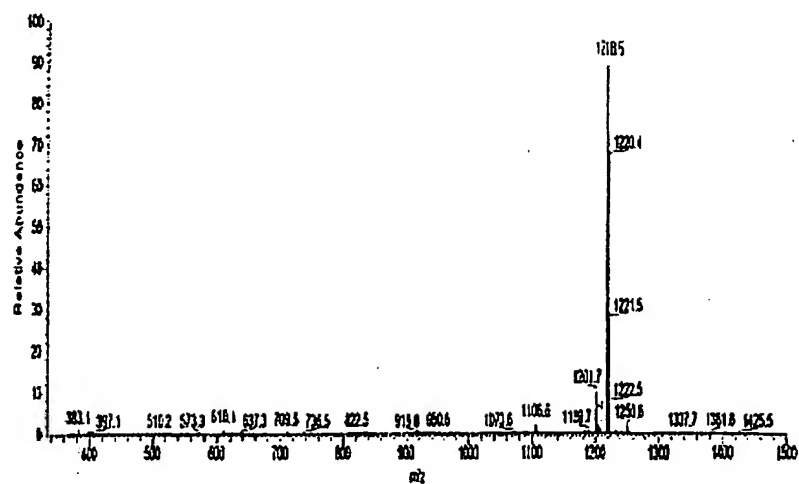
FIG. 4



BEST AVAILABLE COPY

3/17

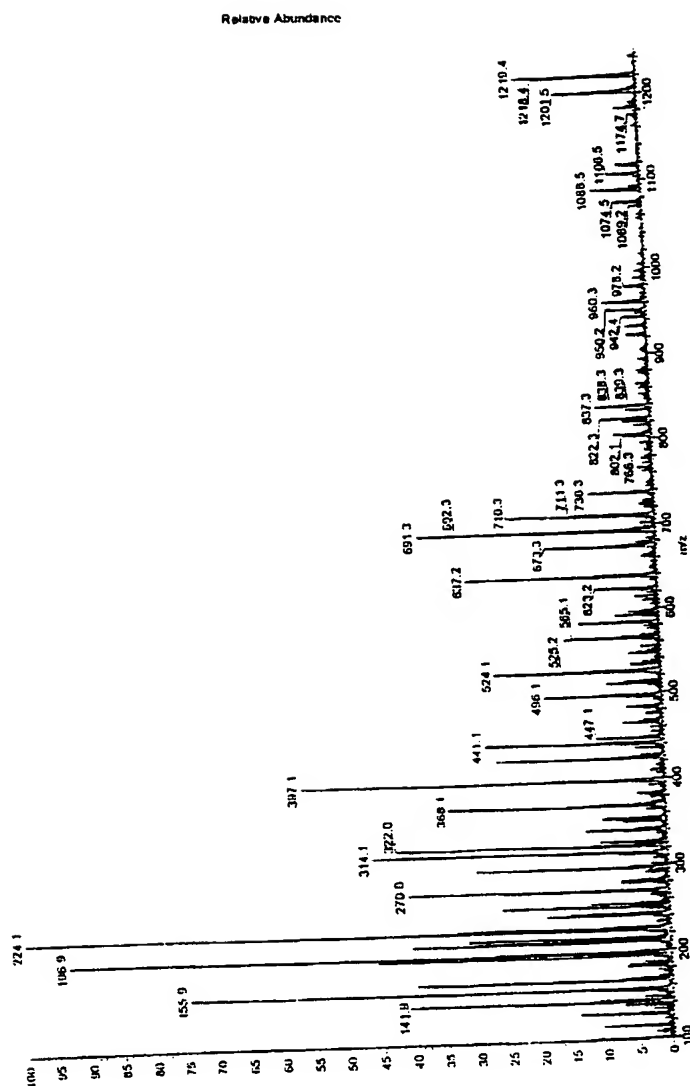
FIG. 5



BEST AVAILABLE COPY

4/17

FIG. 6



BEST AVAILABLE COPY

5/17

FIG. 7

Fragment*	1	2	3	4	5	6	7	8	9	10	11	m/z [Cs A]	m/z [Cs A metabolite]
[M+H] ⁺	0	0	0	0	0	0	0	0	0	0	0	1202.4	1218.5
b ¹⁻¹¹ ₁₀	0	0	0	0	0	0	0	0	0	0	0	1089.6	1105.5
b ¹⁻¹¹ ₉	0	0	0	0	0	0	0	0	0	0	0	982.5	978.4
b ¹⁻¹¹ ₈	0	0	0	0	0	0	0	0	0	0	0	835.4	851.3
b ¹⁻¹¹ ₇	0	0	0	0	0	0	0	0	0	0	0	693.3	709.3
b ²⁻³ ₉	0	0	0	0	0	0	0	0	0	0	0	934.3	950.3
b ²⁻³ ₈	0	0	0	0	0	0	0	0	0	0	0	821.3	837.3
b ²⁻³ ₇	0	0	0	0	0	0	0	0	0	0	0	694.3	710.3
b ²⁻³ ₆	0	0	0	0	0	0	0	0	0	0	0	567.2	583.2
b ²⁻³ ₅	0	0	0	0	0	0	0	0	0	0	0	425.1	441.1
b ²⁻³ ₄	0	0	0	0	0	0	0	0	0	0	0	298.1	314.1
b ³⁻⁴ ₃	0	0	0	0	0	0	0	0	0	0	0	198.9	214.9
b ³⁻⁴ ₂	0	0	0	0	0	0	0	0	0	0	0	637.3	637.2
b ⁵⁻⁶ ₁	0	0	0	0	0	0	0	0	0	0	0	524.2	524.2
b ⁵⁻⁶ ₀	0	0	0	0	0	0	0	0	0	0	0	397.1	397.1
b ¹⁻¹¹ ₁₀ -18	0	0	0	0	0	0	0	0	0	0	0	1071.4	1087.5
b ¹⁻¹¹ ₉ -18	0	0	0	0	0	0	0	0	0	0	0	944.3	961.2
b ¹⁻¹¹ ₈ -18	0	0	0	0	0	0	0	0	0	0	0	675.3	691.2
b ¹⁻¹¹ ₇ -18	0	0	0	0	0	0	0	0	0	0	0	548.3	564.1
b ¹⁻¹¹ ₆ -18	0	0	0	0	0	0	0	0	0	0	0	449.1	465.2
b ¹⁻¹¹ ₅ -18	0	0	0	0	0	0	0	0	0	0	0	322	322

BEST AVAILABLE COPY

6/17

FIG. 8

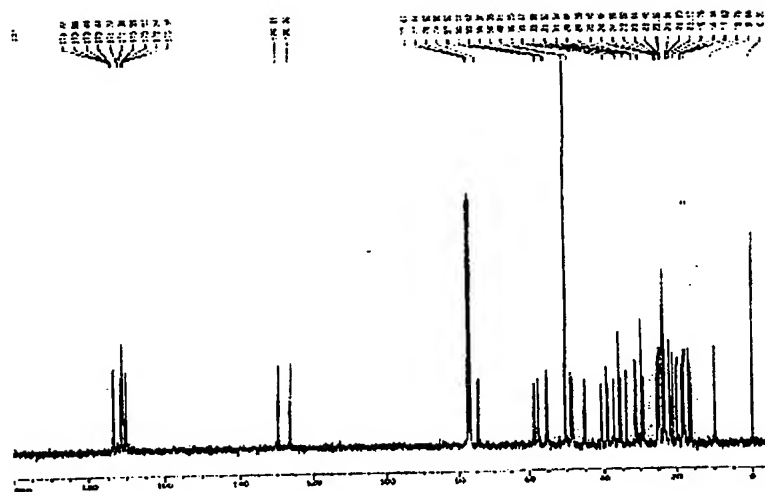
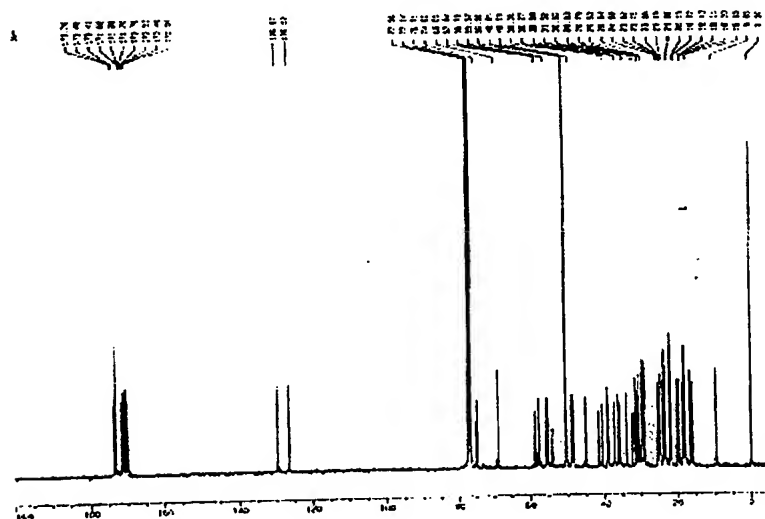


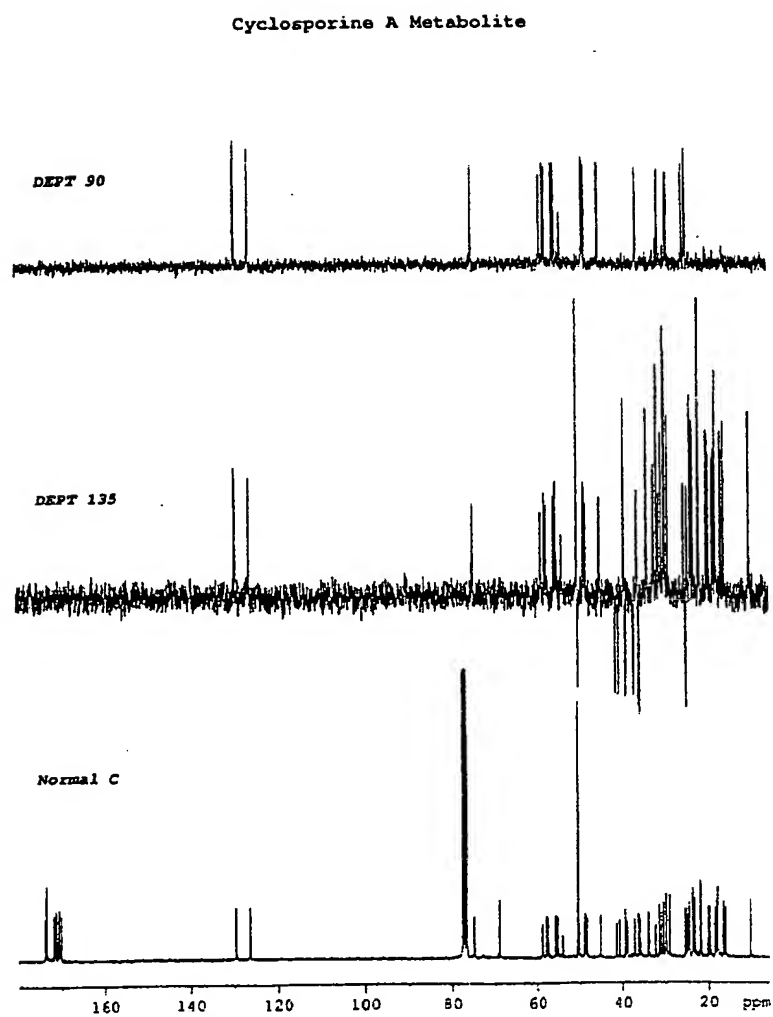
FIG. 9



BEST AVAILABLE COPY

7/17

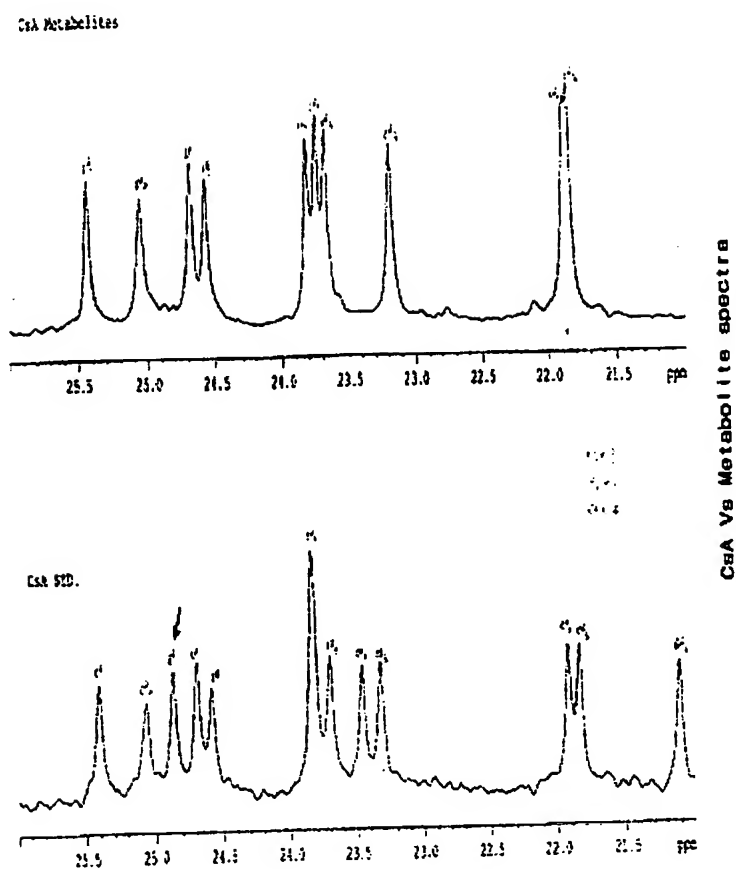
FIG. 10



BEST AVAILABLE COPY

8/17

FIG. 11

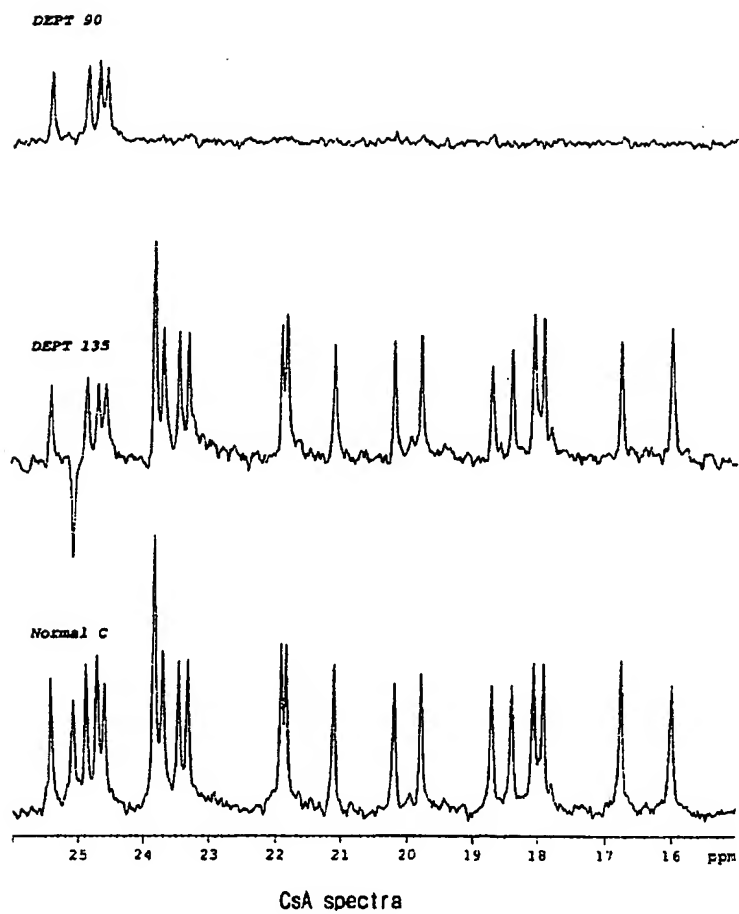


BEST AVAILABLE COPY

9/17

FIG. 12

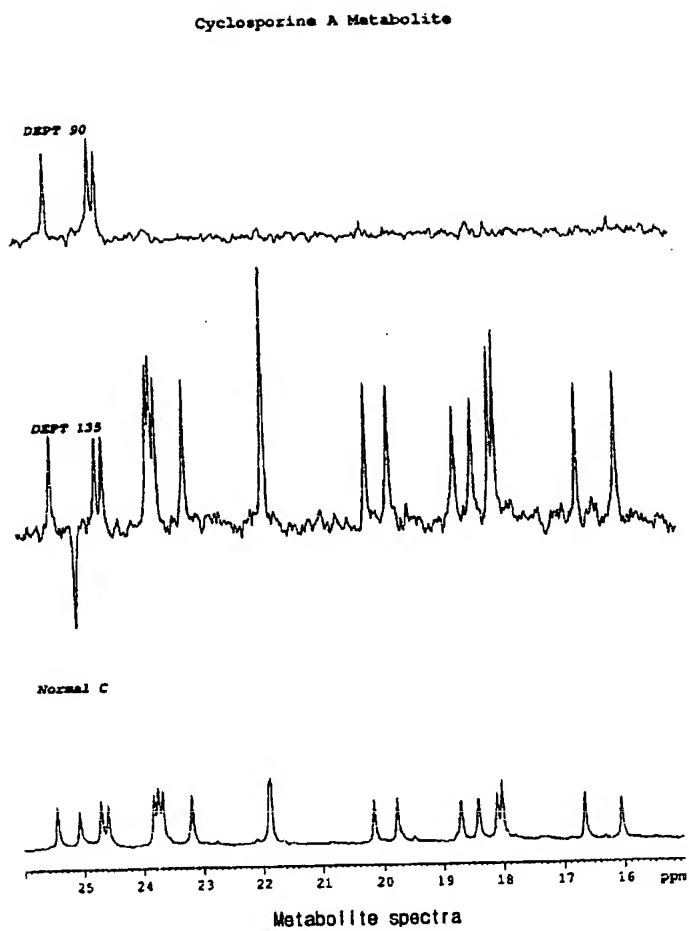
Cyclosporine A STD.



BEST AVAILABLE COPY

10/17

FIG. 13



BEST AVAILABLE COPY

11/17

FIG. 14

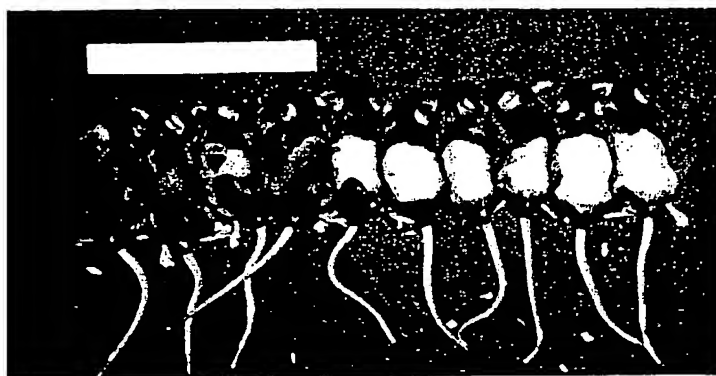
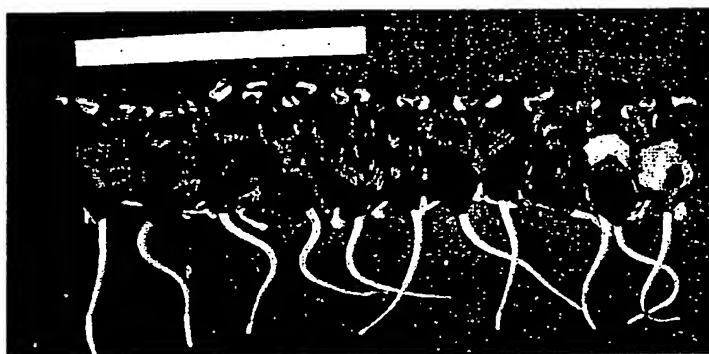


FIG. 15



BEST AVAILABLE COPY

12/17

FIG. 16

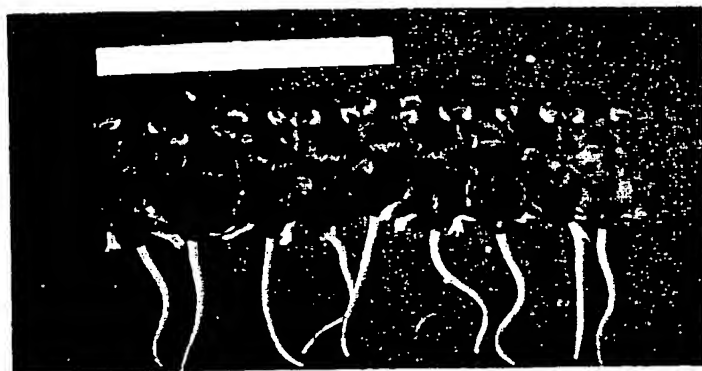


FIG. 17

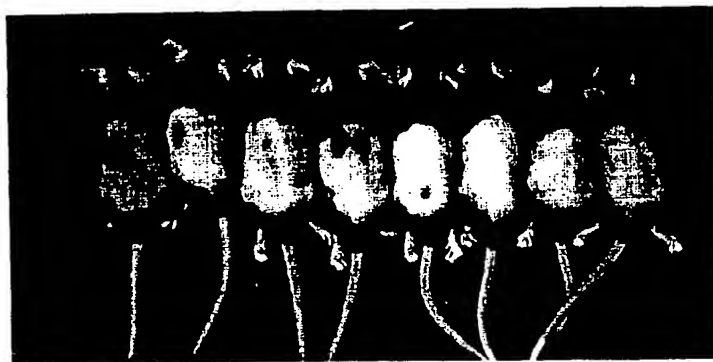


FIG. 18



13/17

FIG. 19

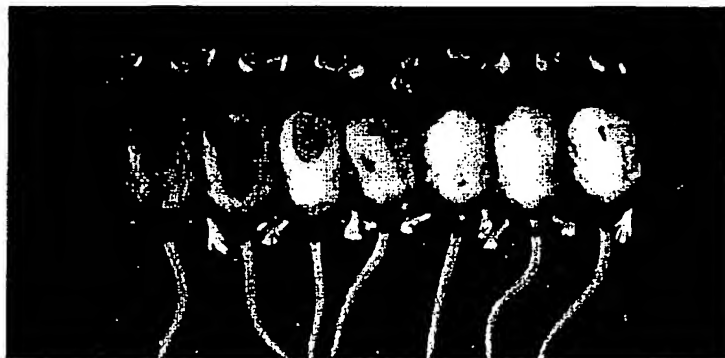


FIG. 20

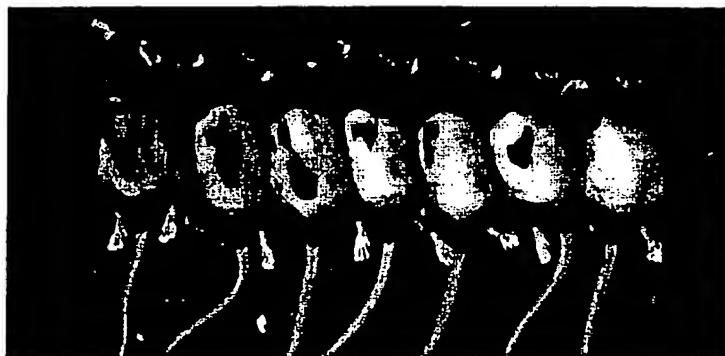
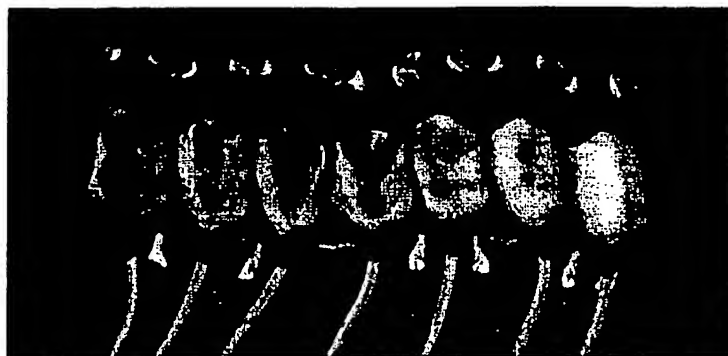


FIG. 21



BEST AVAILABLE COPY

14/17

FIG. 22

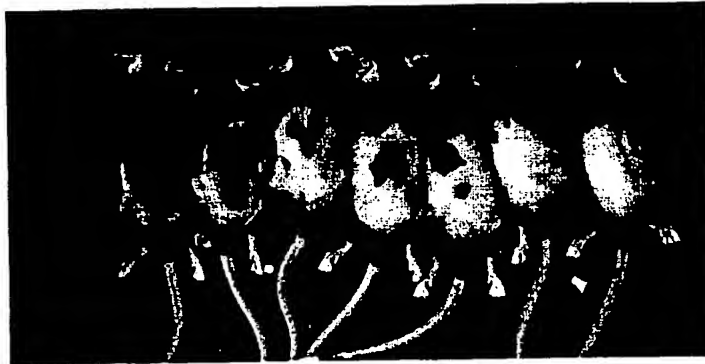


FIG. 23



BEST AVAILABLE COPY

15/17

FIG. 24



FIG. 25



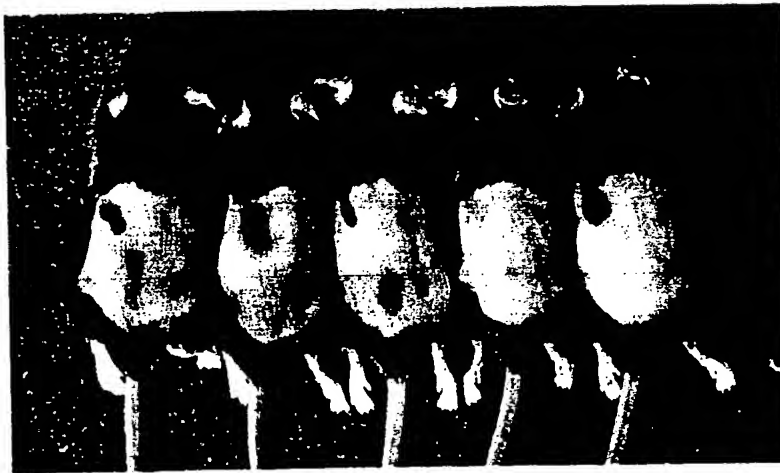
BEST AVAILABLE COPY

16/17

FIG. 26



FIG. 27



BEST AVAILABLE COPY

17/17

FIG. 28



FIG. 29



REST AVAILABLE COPY

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR00/01281

A. CLASSIFICATION OF SUBJECT MATTER		
IPC7 A61K 7/06		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
IPC7 A61K 7/06		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Korean Patents and applications for inventions since 1975		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
CAPLUS (STN), NPS		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MAURER et al., 'Hair growth modulation by topical immunophilin ligands: induction of anagen, inhibition of massive catagen development, and relative protection from chemotherapy-induced alopecia', Am. J. Pathol., American Society for Investigative Pathology, US, 1997, 150(4), p 1433-1441	1 - 2
A	YAMAMOTO et al., 'Hair growth-stimulating effects of cyclosporin A and FK506, potent immunosuppressants, J. Dermatol. Sci., 1994, 7(Suppl.), S47-S54	1 - 2
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
<p>* Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p>		
Date of the actual completion of the international search		Date of mailing of the international search report
15 FEBRUARY 2001 (15.02.2001)		16 FEBRUARY 2001 (16.02.2001)
Name and mailing address of the ISA/KR		Authorized officer
Korean Industrial Property Office Government Complex-Taejon, Dunsan-dong, So-ku, Taejon Metropolitan City 302-701, Republic of Korea		KANG, Choon Won
Facsimile No. 82-42-472-3556		Telephone No. 82-42-481-5608